PATENT COOPERATION TREATY

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From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/000120	06.01.2005	09.01.2004

International Patent Classification (IPC) or both national classification and IPC
A23L1/229, C12P19/30

Applicant
DSM IP ASSETS B.V.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/000120

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10,15
	No:	Claims	11-14,16-18
Inventive step (IS)	Yes:	Claims	1-10
	No:	Claims	11-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	-

2. Citations and explanations

   see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item IV

Lack of unity of invention

U. UNITY (Rule 13.1 PCT).

U.1 The common concept, which would link the processes of claims 1-10 with the compositions of claims 11-18, is the idea of providing a ribonucleotide composition for use as flavouring agent in food, beverage and feed products.

U.1[a] This concept is not novel in view of any of D1-D4 because these documents discloses yeast extracts containing flavouring ribonucleotides for the food/beverage industry (see points 1.1-1.4 below) (see also: D1, column 5, lines 23-26; D3, page 3, lines 10-21; D4, page 30, first paragraph).

U.1[b] Hence, the concept above cannot be considered a general inventive concept according to Rule 13.1 PCT.

U.2 The compositions of claims 11-18 and the processes of claims 1-10 are further characterized by means of different technical features, namely the minimal ribonucleotide content of the compositions and, for example, the separation of the RNA-containing cell wall fraction from the autolysate. None of these composition/process features is present in the definition of the claims of the other category, and therefore the claimed processes do not inherently results in the claimed compositions (see also the PCT Guidelines 10.18 and 10.19).

U.2[a] In view of the different features and the cited prior art, there is no single technical relationship between the processes of claims 1-10 and the compositions of claims 11-18 involving one or more of the technical features to which an inventive step could be addressed (Rule 13.2 PCT).

U.3 Hence, a lack of unity "a posteriori" is indicated, and there is no single general inventive concept between the following separate inventions or groups of inventions:

U.3[a] Subject 1: claims 1-10.

Processes for the production of compositions containing 5'-ribonucleotides by converting the RNA in the RNA-containing cell wall fractions, which have been separated after microorganism autolysis, into said ribonucleotides.

U.3[b] Subject 2: claims 11-18.
Compositions comprising 5'-ribonucleotides and their uses in food, beverages and feed products.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. DOCUMENTS and ABBREVIATIONS.

The following documents are referred to in this communication:
D1: US 4303680;
D2: EP 0354610 A;
D3: EP 1080645 A;
D4: Sommer R., *Lebensmitteltechnik* (1984) vol. 16, no. ½, pages 30-33;
D5: WO 03/063614 A;
D6: WO 03/063613 A;
D7: EP 0299078 A;
D8: *Food Engineering* (1989) Vol. 61, No. 9, Pages 46-48.

5'-XMP (X = G, A, I, C, or U) means guanine-, adenine-, inosine-, cytosine-, or uracil-mono phosphate; **GMP** means mono sodium glutamate.

1.1 D1 discloses yeast extracts containing flavouring ribonucleotides produced by means of yeast autolysis and RNA degradation with phosphodiesterase and, eventually, AMP-deaminase (see the abstract and the examples 1 and 2). The nucleotide content of the extracts is improved by carrying out the autolysis under "controlled" conditions, which leave intact most of the RNA in the lysed cell material, and by extracting this RNA from the lysed cell suspension with a thermal treatment (see lines 31-57 on column 3). The yeast extracts thereby produced contain up to 3.55% of 5'-GMP and 0.7% 5'-IMP, which are the relevant nucleotides with flavouring properties (see lines 31-36 on column 1, table 1 and the last paragraph of example 2).

1.2 D2 discloses yeast extracts containing ribonucleotides for imparting and reinforcing the flavour of foodstuffs (see abstract and lines 42-57 on column 5). In particular, D2 discloses methods for producing yeast extracts with an high content of flavouring ribonucleotides by enzymatically degrading the yeast cells, or the RNA thereof, under oxidizing conditions (see lines 5-15 on column 2). The yeast extracts obtained in accordance with these methods comprise up to 15% of 5'-GMP, calculated on the dry extract (see lines 23-30 on column 5). In one embodiment, the thermally inactivated yeast cells are contacted with malt rootlet suspensions or phosphodiesterase and protease at the same time (see examples 1-4). In an other embodiment, the yeast cell are separately subjected to a first protease-assisted degradation step preceding the thermal inactivation, and to a successive RNA-degradation step with malt rootlet enzymes and, eventually, AMP-deaminase (see examples 5 and 6). These specific processes results in yeast extracts which contain up to 3.4% of 5'-GMP and up to 3.3% 5'-IMP (see examples 5 and 6).

1.3 D3 discloses yeast extracts containing 5'-XMPs, each in an amount of 1-15%, and sodium glutamate as sweetness improving agents (see abstract and examples 1, 11, 15 and 16). In a specific embodiment, the yeast extract is produced by means of a two-step enzymatic degradation of the yeast cells, wherein each step consists of: (i) heating the yeast cell mixture at 90°C for 30 minutes, and (ii) contacting said mixture with proteases or phosphodiesterases for degradation (see example 11).

1.4 D4 discloses yeast extracts obtained by autolysis and teaches that the flavour enhancing properties of these extracts are due to the ribonucleotide content, especially the content of 5'-GMP and 5'-IMP (see page 30: from the third paragraph of the left-hand column to the second paragraph of the central column, and lines 5-20 of the right-hand column). In particular, the flavouring properties of 5'-GMP are better than the ones of 5'-IMP (see page 30, right-hand column, last sentence). In addition, D4 discloses the synergistic effects of the ribonucleotides in combination with amino acids and, specifically, the glutamate salts (see the last paragraph of page 30).

1.5 D5 discloses the addition of a yeast extract to a beverage for the purpose of improving its taste, e.g. the intrinsic vegetable, fruity and alcoholic aroma (see the abstract). In particular, the yeast extract contains up to 50% of total 5'-

ribonucleotides, preferably 5'-GMP, and MSG (see: page 4, lines 5-14; page 5, lines 3-4).

1.6 D6 discloses the use of yeast extracts to improve the organoleptic properties of artificial sweeteners, e.g. for reducing the undesired side- and/or after-taste note (see the abstract). In particular, the suitable yeast extracts contain up to 50% of total 5'-ribonucleotides, preferably 5'-GMP, and MSG (see: page 4, lines 26-33; page 5, lines 1-4 and 27-28).

1.7 D7 discloses yeast extracts for the preparation of seasoning material containing 5'-GMP and 5'-IMP in large amounts (see abstract). In some specific examples, the powdery extracts contains from 32% to 38% of total 5'-ribonucleotides (see examples 3-5).

1.8 D8 suggests the use of yeast extracts containing 5'-ribonucleotides for improving the taste of food products with low or reduced fat content (see the paragraph joining the middle and the right-hand columns on page 46 and the paragraph joining the left-hand with the middle columns on page 47).

2. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

2.1 Claims 1-10 relate to processes for the preparation of compositions containing 5'-ribonucleotides as food or feed additives. Claims 11-18 relate to flavouring compositions and their use in the manufacture of food and beverages. Said processes, compositions and their uses are industrially applicable according to Article 33(4) PCT.

3. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT).
(Invention 1)

3.1 D1 (see point 1.1 above) discloses a process for the production of compositions comprising flavouring ribonucleotides by means of (i) autolysis of yeast cells and (ii)

degradation of the RNA to ribonucleotides. In particular, D1 focuses on the fact that most of the RNA remains not decomposed in the lysed cells after autolysis. The production process of D1 takes advantage from this fact: an improved amount of flavouring ribonucleotides is obtained by means of a thermal treatment, which aims to extract this RNA from the lysed cells, before the insoluble residue (i.e. the cell wall fraction) is discharged.

3.2 D2 and D3 (see points 1.2 and 1.3 above) also disclose processes for producing yeast extracts with improved ribonucleotide content by degrading RNA after a first (proteolytic) degradation step of the yeast, but do not explicitly refer to the fact that the RNA mainly remains not decomposed after the first degradation step, nor that it has to be "extracted" in order to perform its successive degradation into ribonucleotides.

3.3 The claimed process differs from the processes of D1, D2 and D3:

(i) in the separation of the insoluble cell wall fraction, to which the RNA is associated, from the soluble fraction after autolysis, and

(ii) in that the degradation of the RNA into ribonucleotides is carried out on the isolated cell wall fraction, in the absence, for example, of the soluble products from the protein degradation (these proteolysis products are instead present at the RNA degradation step in the processes of the prior art).

3.4 In view of these differences, the process of claim 1 is novel over the prior art, and the problem to be solved can be regarded as the provision of an alternative process for producing compositions with an improved content of flavouring ribonucleotides.

3.5 The solution of separating the soluble and the insoluble fractions after autolysis, in order to carry out the RNA degradation on the isolated RNA-containing cell wall fraction, has not been suggested in the prior art for solving the problem posed, nor can be considered a straightforward possibility that the skilled person would have considered in order to solve the problem posed. Despite D1 teaches that most of the RNA remains not decomposed in the lysed cells after autolysis, D1 does not specifically indicates that the RNA remains associated with the cell wall debris and can be isolated with these debris in an insoluble fraction. It is not even obvious that

the RNA degradation can take place if the RNA is associated with the cell wall (see the requirement of claim 1 that the RNA should remain in a degradable form). The inventors have found that it is possible (see the absence of any specific procedure between the separation step b) and the conversion step c) in claim 1, as well as in examples 1 and 3 of the present application.

3.5[a] The separation of the soluble fraction results in an enrichment of the RNA content in the mixture subjected to RNA degradation, and therefore in an improved nucleotide content of the product.

3.5[b] Hence, the process of claim 1 involves an inventive step over the prior art.

3.6 The subject-matter of dependent claims 2-10 is also novel and inventive for the same reasons above in view of the claim dependency.

4. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT).
(Invention 2)

4.1 The subject-matter of claim 11 is not novel over the yeast extracts disclosed in D2, D3 and D5-D7 because these extracts contain large amounts of 5'-ribonucleotides, i.e. from 15% to 50% of 5'-XMP as calculated on the dry extract (see points 1.2, 1.3, 1.5-1.7 above).

4.2 Dependent claims 12-13, as well as claims 14 and 16-18, do not appear to contain any additional features which, in combination with the features of any claim to which they eventually refer, meet the requirements of the EPC with respect to novelty and/or inventive step, given the disclosure of the prior art.

4.2[a] In particular, the preferred extracts of D2, D5 and D6 mainly contain 5'-GMP, and D4 teaches that 5'-GMP is to be preferred to 5'-IMP (see point 1.4 above). In addition, the yeast extracts of D3, D5 and D6 are used for improving the organoleptic properties of beverages and artificial sweeteners (see points 1.3, 1.5 and 1.6 above).

4.3 The subject-matter of claim 15 is novel because the available prior art does not disclose the use of yeast extracts containing high amounts of 5'-ribonucleotides for improving the taste of food products with low or reduced fat content. None of D2, D3

and D5-D7 is specifically directed to low or reduced fat foods, and D8 does not disclose a composition containing high amounts of 5'-ribonucleotides (see points 1.2, 1.3, 1.5-1.8 above).

4.4 Nevertheless, the subject-matter of claim 15 cannot be considered as involving an inventive step because the skilled person would have considered to use the new generation of yeast extracts containing high amounts of 5'-ribonucleotides, like any of the extracts disclosed in D2, D3 and D5-D7, in place of the yeast extract of D8 in order to solve the problem of improving the organoleptic properties of food products with low or reduced fat content.

Re Item VII
Certain defects in the international application

D.1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2, D3 and D5-D8 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

C. CLARITY (Art. 6 PCT).

C.1 The term "substantial" used in claim 1 is vague and leaves the reader in doubt as to the meaning of the part of the RNA, which remains in the degradable/associated form, thereby rendering the definition of the subject-matter of said claim unclear.

C.2 Although claims 14-17 have been drafted as separate independent claims, they appear to relate to the use of the claimed composition as a food or feed additive, i.e. they effectively relate to the same subject-matter and differ from each other only with regard to the definition of preferred features. The aforementioned claims therefore lack conciseness and as such do not meet the requirements of Article 6 PCT.